ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

CM12 USER GUIDE

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Centre Interdisciplinaire de Microscopie Electronique

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The Electron Microscopy Unit at the University of Sydney is gratefully acknowledged for providing some of the source material.

CM12 START UP

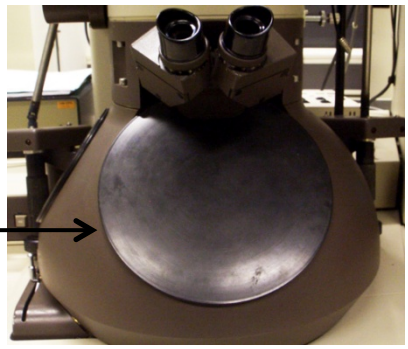
- **Log in to PC computer.**

RIGHT HAND CONTROL PANEL

- Push in **PANEL DIM** button, turn **DATA DIM** button clockwise. Panel lights and CRT display should light up.

- **DO NOT TOUCH the Microscope ON, STANDBY or OFF buttons!**

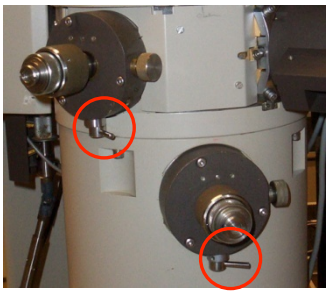
- Fill the **cold trap** with liquid N_2.
  Note cold trap **must be refilled** after 3-4 hours.
  Leave **screen cover** on when filling the cold trap!



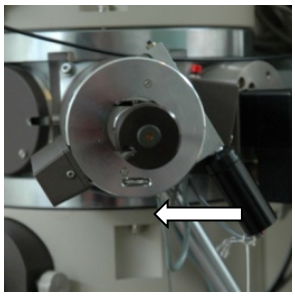
CM12 START-UP CHECK LIST

- **CCD camera control** (left of microscope column):
  Power on, PELTIER COOLER: COOL,
  SHUTTER: AUTO, CAMERA: OUT.

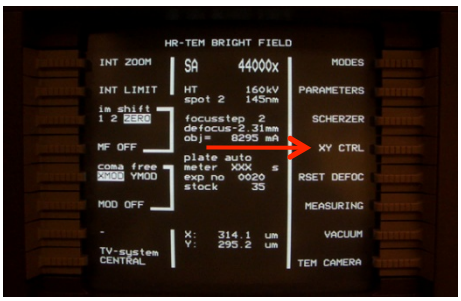
- On microscope column, **Objective aperture** and **Selected Area aperture** out (levers to the right).



- Manual tilt on sample goniometer is at **0° tilt**; manual tilt control of the goniometer is fixed – black lever pushed towards the unit.



- Check stage positions **X: ~0.0 um, Y: ~ 0.0 um**
  If not, select **XY CTRL** to access XY control page; then press **RESET** button, just below the CRT screen on the left, to zero X, Y.
  Once reset complete, press **READY** button, just below CRT screen on the right, to return to main CRT page.



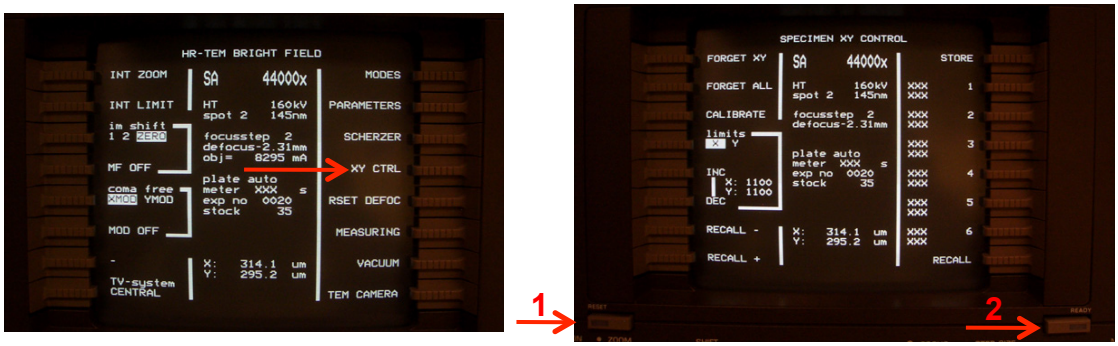
SPECIMEN EXCHANGE

- On microscope column, check **Objective** and **Selected Area** apertures out (levers to right – see p. 28) and that **cold trap** is filled with liquid N_2.

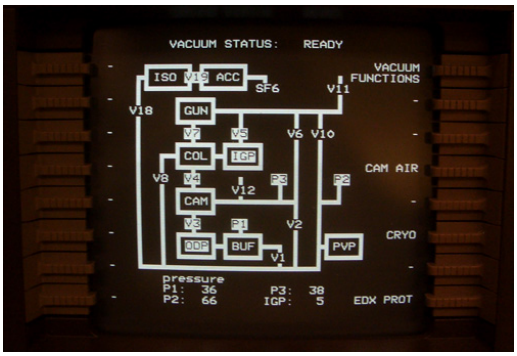
CRT DISPLAY

- Check stage positions **X: ~0.0 um, Y: ~ 0.0 um.**

  If not, select **XY CTRL** to access XY control page; then press **RESET** button, just below the CRT screen on the left, to zero X, Y.
  Once reset complete, press **READY** button, just below CRT screen on the right, to return to main CRT page.



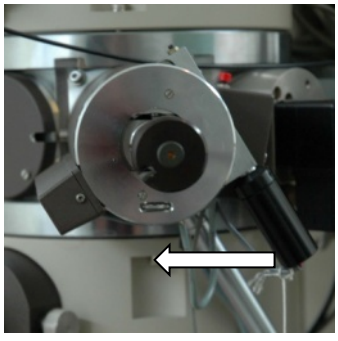
- Select **VACUUM** for VACUUM STATUS page.
  Check status is READY and IGP < 23.



SPECIMEN HOLDER

- Check manual (α) tilt is **0°**, and that manual tilt control of the goniometer is fixed by pushing black lever towards the unit.

  If you have used **Double Tilt (DT)** holder, adjust β tilt to intermediate position suitable to the specific holder.



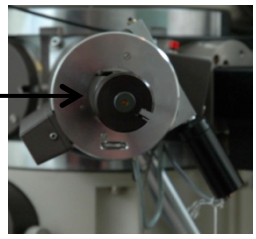
REMOVING SAMPLE HOLDER

**** REVIEW ALL SPECIMEN REMOVAL STEPS BEFORE PROCEEDING. IT IS CRITICAL THAT THE FOLLOWING PROCEDURES BE FOLLOWED EXACTLY. ****

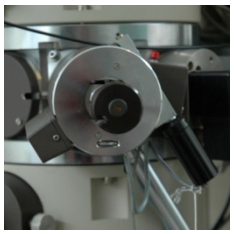
- Put on gloves.

- With one hand, firmly grip the handle of the specimen holder and pull holder back as far as it will go. **DO NOT ROTATE WHILE PULLING BACK.**

  When the holder reaches the stop, rotate clockwise 3 to 5 degrees and gently let go. The holder should now be sitting on a ledge and will not be drawn in, if it does get drawn in then repeat procedure and rotate clockwise a little further.



- Now continue to gently rotate holder clockwise until it reach the next stop.



- Withdraw holder from microscope in a **controlled method.**

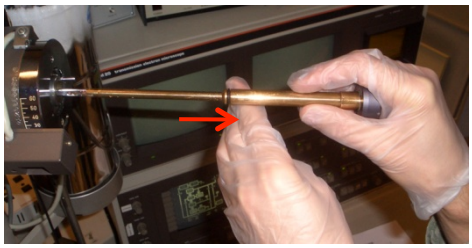
Ask for a demonstration if required

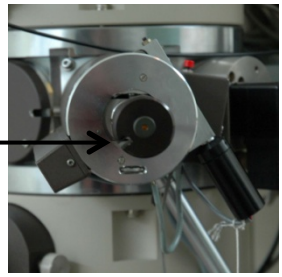
INSERTING SAMPLE HOLDER

**** REVIEW ALL SPECIMEN INSERTION STEPS BEFORE PROCEEDING. IT IS CRITICAL THAT THE FOLLOWING PROCEDURES BE FOLLOWED EXACTLY. ****

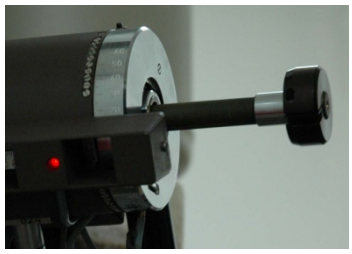
- On CRT screen, check stage positions **X: ~0.0 um, Y: ~ 0.0 um** and **IGP < 23.**
- Wear/put on gloves. Turn on flow in laminar flow box.
- Load your sample into specimen holder. *Never touch the sample side of the o-ring with a bare hand!*
- Using optical microscope in laminar flow box, check the o-ring on the specimen holder for dust. **If dust found, carefully remove it with a tooth-pick.**
- Align the specimen holder so that the pin sticking out is pointing towards 3 o'clock. One good practise is to rest the specimen holder rod on the index finger of the left hand:

Note index finger to ***right*** of o-ring.





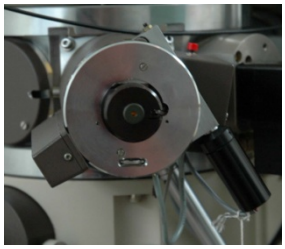
- Gently insert the holder until pin contacts and holder cannot be pushed in any further. Now gently turn the holder clockwise until it finds the slot and slides further. IT MUST BE IN THIS SLOT!



- You should have heard the vacuum system come on and the **RED LED** on the goniometer lights up.

Do not rotate holder anticlockwise until RED light turns off!

- When **RED LED** on the goniometer turns off, gently rotate the holder anticlockwise until the holder is drawn in by the microscope vacuum.



- On **VACUUM STATUS** page of CRT display, **IGP** may increase temporarily to ~30-40, but should quickly recover to < 23.

SELECTING HT

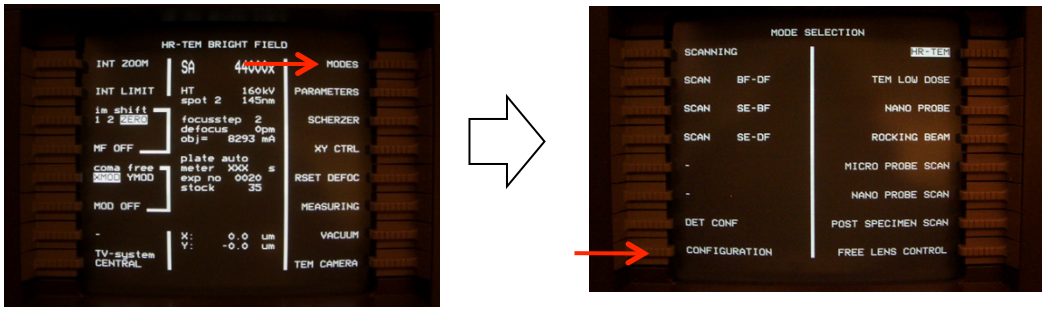
RIGHT HAND CONTROL PANEL

- HT should be left on all the time. However *if* HT OFF press **HIGH TENSION ON/OFF** to turn on HT (green light comes on).

- On main CRT page select **PARAMETERS** page
  Set HT as desired – 80/100/120 kV
  Recommendations:
  - Materials science work 120 kV (default)
  - High contrast bio-TEM: 80 kV

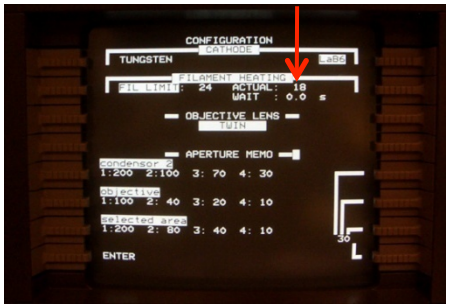
STARTING THE FILAMENT

- On **VACUUM STATUS** page check or wait until **IGP < 23** and **VACUUM STATUS: READY**. Once **IGP < 23**, press **READY** button to return to main CRT page.
- On main CRT page select **MODE** to go to MODE SELECTION page
  On MODE SELECTION page select **CONFIGURATION** to CONFIGURATION page



RIGHT HAND CONTROL PANEL

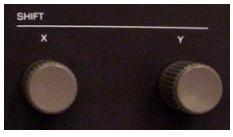
- Start turning the **FILAMENT** knob clockwise by one click every 20 seconds, until **ACTUAL** value on CRT display is about **6-7 less** than the **FIL LIMIT** value.*



- Remove the cover from the glass viewing window; **find the beam** (see “FINDING THE BEAM”, p. 29). Move sample so that beam is in vacuum or on very thin part of specimen.
- Once beam is found, increase **MAGNIFICATION** to **19500x** using dial on RHS panel.

- Focus this beam to its smallest diameter using the **INTENSITY** dial. You may need to select **FINE** control to do this; i.e. the button on the left of the **INTENSITY** dial.

- Centre beam in middle of screen using **SHIFT** dials. (RHS panel.)



* If no illumination visible then increase **FILAMENT** by another click and repeat process *

GUN TILT ALIGNMENT

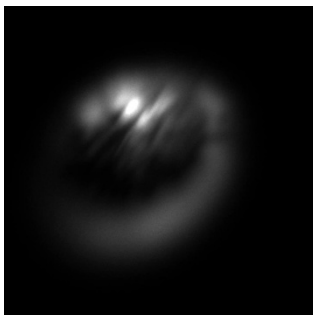
The aim is to try and make the intensity of the halo approximately symmetrical.

- Examine image of focused beam, does it show a halo surrounding a bright spot? If not try increasing or decreasing the **FILAMENT** by one click until a halo is visible.

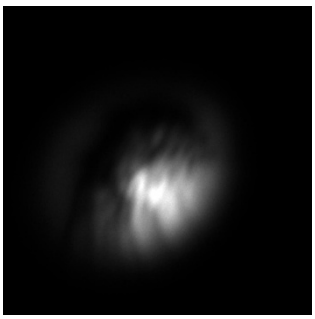
- Press **ALGN** button in the **MULTFUNCTION** controls to enter ALIGNMENT page on CRT. (RHS panel)

- Then select **gun TILT** on the CRT screen.

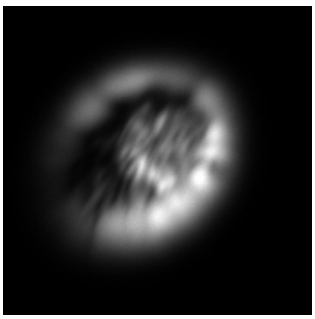
- Using the **MULTIFUNCTION** dials **X and Y** make the halo intensity approximately symmetrical. (This may require you to experiment a bit to see the effect of each dial.)



Poor Gun Tilt

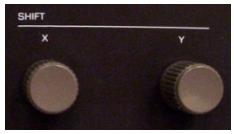


Poor Gun Tilt



Good Gun Tilt

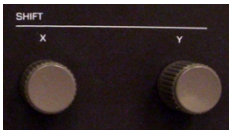
If necessary re-centre beam back to the centre of screen using beam **SHIFT** dials. (RHS PANEL)



CONDENSER ASTIGMATISM

The aim is to make the fine structure in the image of the beam as small and sharp as possible

- Check that beam is centred in middle of screen using beam **SHIFT** dials.



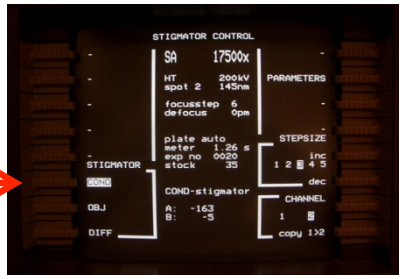
- Raise the small viewing screen by lifting the back lever on LHS of viewing screen and observe the fine structure in the filament image using the binoculars.

  * You may need to focus the binoculars; if so, insert the beam stop (see p. 26) & focus binoculars on its shadow. *

- Refocus to give the clearest image using the **INTENSITY** dial. You may need to select **FINE** control.

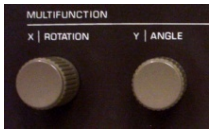
- Press the **STIG** button in the **MULTIFUNCTION** controls.

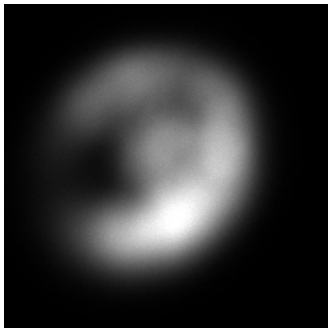
- Check **COND** is selected in **STIGMATOR CONTROL** page on CRT.



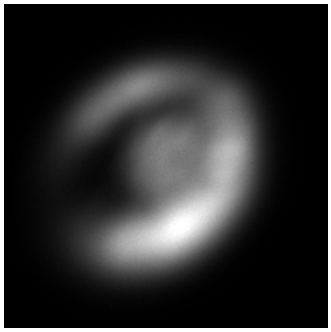
- Adjust the **MULTIFUNCTION X and Y** dials to make the fine structure in the image as sharp as possible.

  Do not turn X and Y at the same time. Instead, turn one to give the sharpest possible image, then the other, and repeat.

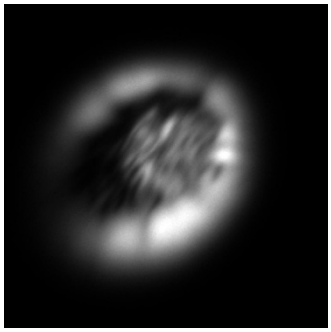




Poor Cond. Stig.



Poor Cond. Stig.



Good Cond. Stig.

- After adjusting the condenser astigmatism, try refocusing beam with **INTENSITY** and repeat condenser astigmatism correction.
- Switch off **STIG** and **ALGN** buttons when finished.

FILAMENT SATURATION

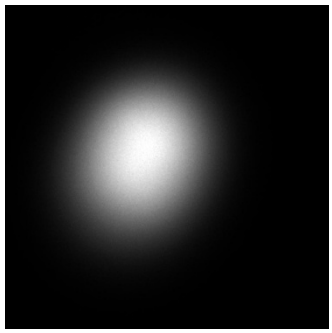
The aim is to remove all fine structure or shadows from the beam.

- With all **MULTIFUNCTION** buttons switched off you should have returned to the **CONFIGURATION** page on the CRT. The beam should still be focused to the smallest diameter spot. The temptation now is to just increase the filament until the **FIL LIMIT** is reached; *however, this is not a good practice*.

- To saturate the filament correctly, increase the **FILAMENT** dial by one click and wait the prescribed 20 seconds.

- Check image on screen and see if all the fine structure or shadows have disappeared.

- If fine structure or shadows still exist then increase **FILAMENT** dial by one click and repeat process. Repeat until all fine structure or shadows disappear.
  The filament is now saturated. Its image should look like this:



- Even if **FIL LIMIT** is not reached, once saturated do not continue increasing **FILAMENT** current. Doing so is called oversaturation, and is very bad for filament lifetime.

- If the filament is almost, but not quite, saturated when the **FIL LIMIT** is reached, continue with subsequent microscope alignments and see if the fine structure or shadows disappear after another 15 to 30 minutes, as the filament warms up. If not, contact one of the microscope responsibles.

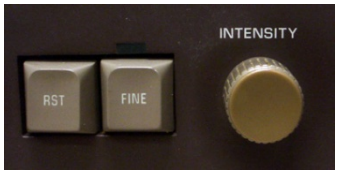
- About 30 minutes after first saturation of the filament, try decreasing **FILAMENT** by one or two clicks and checking the focused beam for fine structure, to see if the correct saturation current has decreased slightly as the filament has warmed up.

CONDENSER APERTURE CENTRING

The aim is to have the beam converge and diverge concentrically; if the **CONDENSER APERTURE** is not centred correctly then the beam will not converge and diverge concentrically when the **INTENSITY** dial is altered.

- Have **MAGNIFICATION** set at 19500x.

- Select the desired **CONDENSER APERTURE** size – see p. 28 on “HOW TO USE APERTURES”.
  (For imaging, usually use second largest aperture.)
- Focus the beam to its smallest diameter using the **INTENSITY** dial.



- Centre the focused beam in middle of screen using **SHIFT** dials.

- Turn **INTENSITY** dial clockwise to form an overfocus spot around ¾ the diameter of the screen.
- Re-centre the beam with the aperture controls.

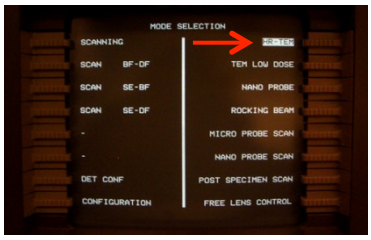
Repeat method if the aperture centre was found to be a long distance out.

FINE GUN TILT & CONDENSER ASTIGMATISM

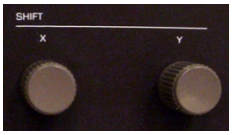
Gun tilt: Optimizing brightness of the beam.

NOTE ON CM12 FILAMENT NORMALLY MOVES DURING USE. THEREFORE GUN TILT MAY HAVE TO BE REPEATEDLY OPTIMISED IN THIS WAY DURING YOUR SESSION!!!

- After reaching FIL LIMIT, press **READY** button just below CRT screen on the right to return to **MODE SELECTION** page. Then select **HR-TEM** to return to main CRT page.

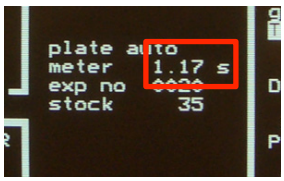


- Turn **INTENSITY** dial clockwise to form an overfocus spot about 6-7 cm in diameter. Centre beam in middle of screen with **SHIFT** dials.



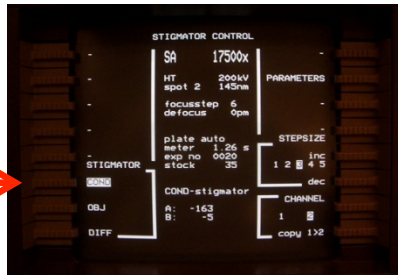
- Press **ALGN** button in the **MULTIFUNCTION** controls again, and select **gun TILT** on the CRT screen.

- Use the **MULTIFUNCTION X and Y** dials to minimise the exposure time of the **plate auto meter** on the CRT screen.



Condenser stigmater: Optimizing circularityof the beam.

- Turn **INTENSITY** dial anticlockwise and centre the beam using the **SHIFT** dials so that the beam size and position is matched to the larger black circle printed in the middle of the fluorescent screen.
- Press **STIG** in the **MULTIFUNCTION** controls and check that **COND** is selected on the **CRT** screen.



- The beam should be circular; use printed circle as a guide. If not, use the **MULTIFUNCTION X and Y** dials to make it circular. It should remain circular if you turn **INTENSITY** further clockwise to form an underfocus spot.
- Once beam is circular, and brightness is maximised, switch off **STIG** and **ALGN** buttons.

SPOT SIZE

Changing the spot size allows you both to adjust the intensity of the electron beam, and the diameter of the focused electron beam.

- The spot size currently in use is specified on the CRT main page.

- Spot size is controlled using the **SPOT SIZE** dial on the left of the RHS control panel.

- Default **SPOT SIZE** is **spot 2**

- Turning the **SPOT SIZE** dial clockwise gives a smaller, dimmer spot (higher **spot** number). Turning the dial anticlockwise gives a larger, brighter spot (lower **spot** number).

- For EDX analysis a higher spot number allows you to analyse from a smaller region, but gives you less EDX signal.

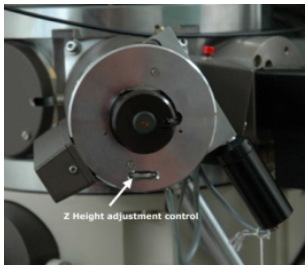
EUCENTRIC SPECIMEN HEIGHT ADJUSTMENT

The aim is to bring the sample to the right height for correct imaging

- Move the stage around until you find your specimen or region of interest.
- Set magnification to 11500x (RHS panel)
- Centre beam in middle of screen using **SHIFT** dials. (RHS panel).

- Turn **INTENSITY** dial clockwise to form an overfocus spot about the size of the screen.
- Move stage around and choose an easily recognizable feature. Place this feature in the middle of the screen.
- Release the manual tilt control by pushing the black lever, situated behind the goniometer, away from the unit.

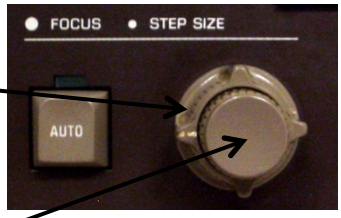
- Start rotating the goniometer unit clockwise and note the direction of movement on the image.
- Now continue rotating the goniometer clockwise until a few degrees tilt. Lock goniometer manual tilt again.
- Adjust the specimen Z height, using the dial in front of the goniometer to bring this feature back to the middle of the screen.



- Release manual tilt control again and rotate the holder back the 0 degrees.
- Repeat these until movement is minimized.

FINDING SPECIMEN FOCUS

- Move stage around and choose an easily recognizable feature.

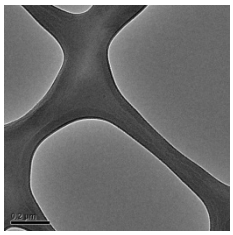


- Turn the **FOCUS** dial (the large, outer dial) to change the focus of the image.

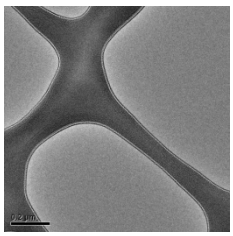
- If the focus only changes by a small, insufficient amount, increase the **FOCUS STEP SIZE** (see **focusstep** number on CRT display) by turning the small, inner dial clockwise to e.g. 5 or 6.

- To refine focus, decrease **FOCUS STEP SIZE** to e.g. 3 or 4 in order to make focus control more sensitive.

- If the specimen is **under focus** (focus turned **anticlockwise**) you will see strong **bright fringes** around the edges of features.

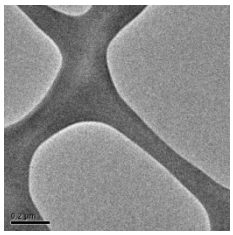


- If the specimen is **over focus** (focus turned **clockwise**), you will see **dark fringes** around the edges of features.



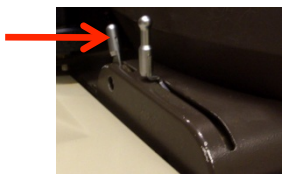
- Absolute focus (between under focus and over focus) is the focus that gives **minimum contrast** (see right).

  Images are normally recorded with **slight under focus**, so that features have enough contrast to be observable, and the images are still sharp.



- If you are having difficulty finding focus, press **AUTO** focus. If you have previously set **EUCENTRIC HEIGHT** correctly this should give focus near to (*but perhaps not exactly at*) absolute focus.

- To use the **binoculars** to see the image more closely, push up the back lever to the left of the viewing window to bring down the small fluorescent viewing screen.



  * If binoculars are out of focus, insert the beam stop (control on RHS of window – see p. 26) and focus the binoculars on its shadow. *

TWO BASIC COLUMN ALIGNMENTS

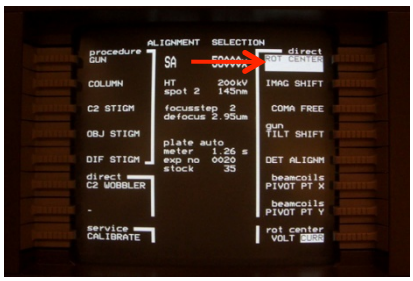
- Press the **ALGN** button in the **MULTIFUNCTION** control (RHS control panel) to access the **ALIGNMENT SELECTION** page on the CRT.

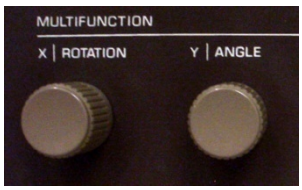
1) ROTATION CENTER

Rotation center alignment is necessary if the image moves when FOCUS is changed. The alignment corrects this movement.

- Move stage around and choose an easily recognizable feature.

- Use beam **SHIFT X and Y** to centre the beam on the main fluorescent screen.
- Increase **MAGNIFICATION** to 53000x. While viewing with the small fluorescent screen and binoculars, centre and focus the recognizable feature.
- Choose **ROT CENTER** on the CRT display. Microscope now wobbles focus back and forwards.





- Adjust **MULTIFUNCTION X and Y** dials to minimise any translation of the image while focus is wobbled.
- When rotation center is well-aligned, image will rotate on-axis as focus is wobbled, but will not translate across the screen.
- To increase sensitivity of rotation center correction, increase the **FOCUS STEP SIZE** of the **FOCUS** control to increase degree of focus wobble.

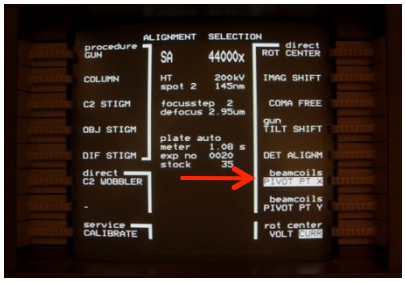
2) PIVOT POINTS

Aim: keep electron beam aligned with optical axis when beam is shifted. This alignment has to be done with a sample that is perfectly eucentric and focussed.

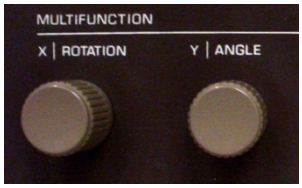
- Set **MAGNIFICATION** to 40000x

- Use beam **SHIFT X and Y** to centre the beam on the fluorescent screen.

- Focus the beam to the smallest, brightest spot possible using **INTENSITY** (LHS control panel). You may need to select **FINE** to make **INTENSITY** dial more sensitive.



- Choose beamcoils **PIVOT PT X** on the CRT display. Microscope now wobbles the beam coils. There are two images of the beam.



- Adjust **MULTIFUNCTION X and Y** dials to coincide the two images of the beam so that they exactly overlap.

- Once **PIVOT PT X** aligned, select **PIVOT PT Y** and repeat the alignment procedure.

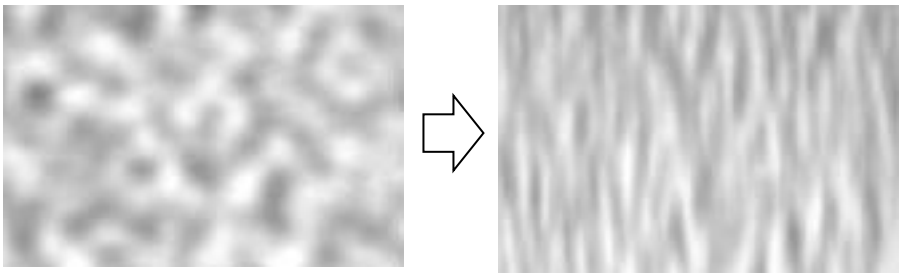
- After both pivot points are aligned, press **ALGN** in the **MULTIFUNCTION** panel to deselect ALIGNMENT page and return to main page on the CRT.

- Re-centre the beam with **SHIFT X and Y.**

- N.B. you may need to repeat or iterate the rotation center alignment after aligning the pivot points.

OBJECTIVE ASTIGMATISM

- Correcting **objective astigmatism** is the last step in microscope alignment for imaging. Minimal **objective astigmatism** is essential for taking sharp, accurate images, particularly at higher magnifications. This correction is also the most difficult alignment, and **requires practise by the user**.
- If stigmatism is poor, the image will be distorted (i.e. round features will not be round):



Good objective astigmatism **Bad objective astigmatism**

- Objective astigmatism can vary day-to-day, throughout the session, and can depend on the sample. Therefore it must be checked regularly.
- There are many possible methods for correcting objective astigmatism. Here we present four different methods:

  A: FFT of amorphous material

  B: Fresnel fringes on small round feature

  C: Off-eucentric convergent beam (crystalline sample)

  Each method may or may not be suitable depending on your sample. Which method or methods you are demonstrated and practise during training will take this in to account. We will be happy to give advice about suitable objective astigmatism correction methods for your work. **Do not hesitate to ask if you would like advice.**

- Trouble-shooting: If you are **having trouble or are completely "lost"**, try setting the objective stigmators to **A: 0**and **B: 0** on the CRT display. These are typically good values.

CORRECTING OBJECTIVE ASTIGMATISM
METHOD A: FFT

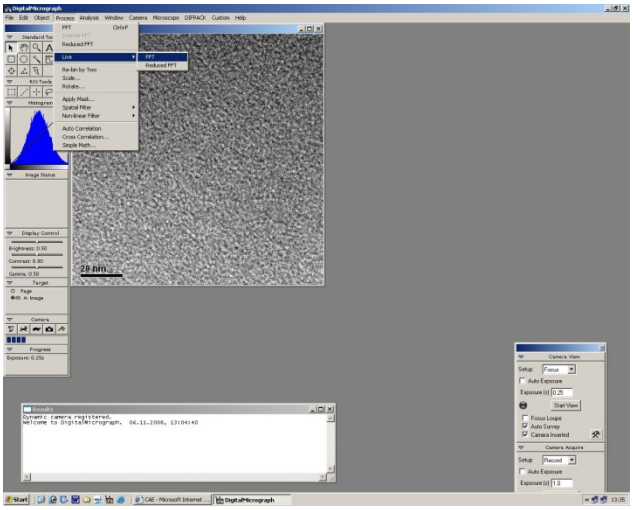
- Find some **amorphous material** on your TEM sample
  – e.g. amorphous carbon support film, glue line for sandwich cross-section, or amorphised layer on edge of crystal left from ion bombardment.

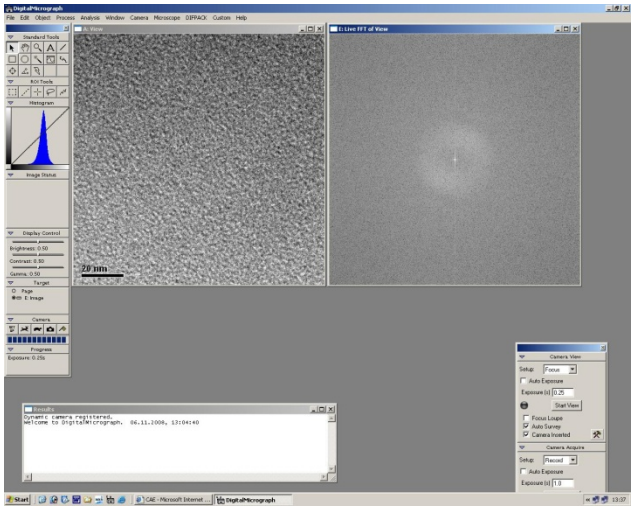
- **FOCUS** the amorphous material, and increase **MAGNIFICATION** to 88000x or 110000x.

- Move stage so that amorphous material is in the centre of the screen. Focus so that you are close to absolute focus (i.e. **minimum contrast**). Centre beam with beam **SHIFT X and Y.** Adjust **INTENSITY** to provide sufficient illumination. You may need to select **FINE** control.

- Start viewing the amorphous area on the **CCD camera** in **Focus** mode (see “USING THE CCD CAMERA” p. 30). Focus mode should be set up for continuous acquisition.

- In the “**Process**” drop down menu in **DigitalMicrograph**, select **Live, FFT**. A window showing a live Fourier transform of the image opens up:

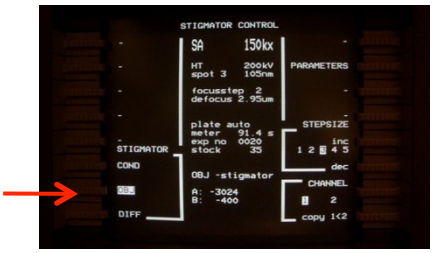




OBJECTIVE ASTIGMATISM METHOD A: FFT cont. 1

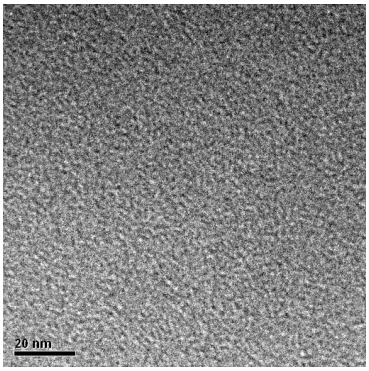
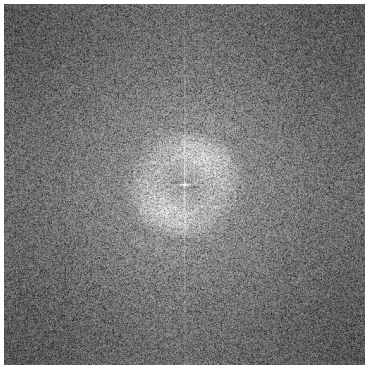
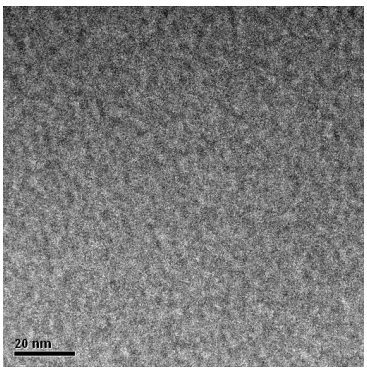
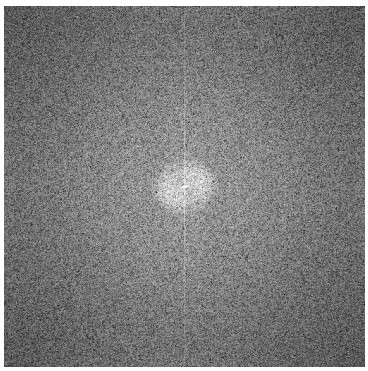
- Press **STIG** in the **MULTIFUNCTION** panel to select the **STIGMATOR CONTROL** page on the CRT.

- Check that **OBJ** is selected on the CRT display. **MULTIFUNCTION X and Y** now control the objective stigmators.



- Select **FOCUS STEP SIZE** of 2 or 3, and adjust **FOCUS** near to achieving minimum contrast.

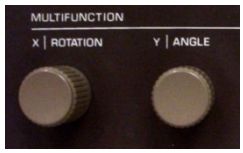
- Look at the **live FFT** image of the amorphous material. Turn **FOCUS** anticlockwise to be **under focus**. You are aiming to see a diffuse ring in the live FFT that contracts as you go more under focus.

	Image	**FFT**
Less underfocus		
More underfocus		

- If you turn **FOCUS** clockwise, the ring will expand and then disappear as absolute focus or minimum contrast is achieved.

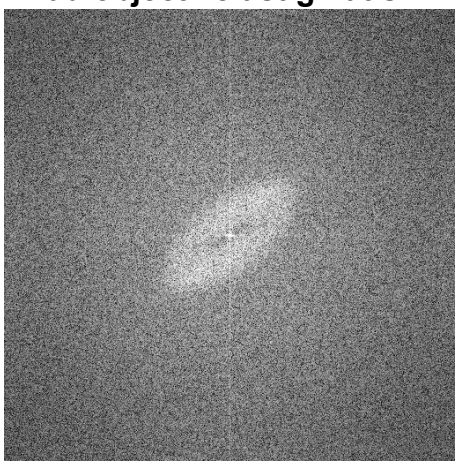
- If you continue turning **FOCUS** clockwise to go over focus, the diffuse ring will reappear and start contracting as you go progressively over focus.

OBJECTIVE ASTIGMATISM METHOD A: FFT cont. 2

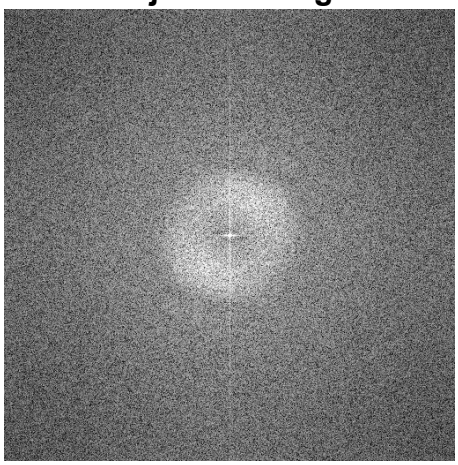


- Go back to **under focus**. If objective astigmatism is present, the diffuse ring will not be circular. Using **MULTIFUNCTION X and Y**, adjust the stigmators until the diffuse ring is **circular.** Turn one dial until minimum astigmatism obtained, then the other, and repeat. Do not turn them both at the same time.

Bad objective astigmatism

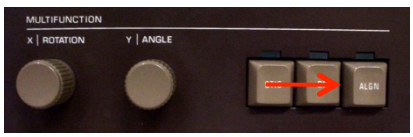


Good objective astigmatism



- To make coarse adjustments to the objective stigmators, go more under focus (smaller diffuse ring) and increase the stigmator **STEPSIZE** to 3 or 4 on the CRT display. To make fine adjustments, go less under focus (larger diffuse ring) and reduce stigmator **STEP SIZE** to 2 or 3.

- Once you have finished correcting objective stigmatism, press **STIG** in the **MULTIFUNCTION** panel to return to the main CRT page.

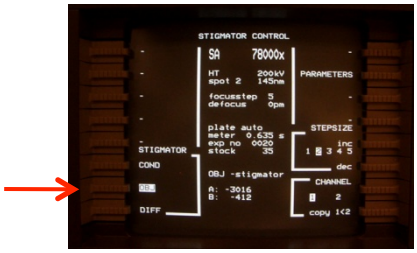


CORRECTING OBJECTIVE ASTIGMATISM
METHOD B: Fresnel fringes

- Choose one detail on your specimen that has a round shape or that is very marked/clear (e.g. a small round hole).
- **FOCUS** the amorphous material, and increase **MAGNIFICATION** to e.g. 66000x.

- Underfocus or overfocus until a bright or dark Fresnel fringe is observable around the edge of the feature.
- For more detailed observation, use the TV camera; see **METHOD A: TV Camera** p. 21-23 for instruction on how to use the camera.
- Press **STIG** in the **MULTIFUNCTION** panel to select the **STIGMATOR CONTROL** page on the CRT.

- Check that **OBJ** is selected on the CRT display. **MULTIFUNCTION X and Y** now control the objective stigmators.



- Turn the **MULTIFUNCTION X and Y** dials to obtain a uniform Fresnel fringe when the image is slightly overfocused (one **FOCUS** click clockwise from focus of minimum contrast with **FOCUS STEP SIZE** of 4). Adjust **X and Y** alternately; turn one until minimum astigmatism observed, then the other. Do not try to adjust both dials at the same time.
- To make coarser adjustments to the objective stigmators, go more under focus (smaller diffuse ring) and increase the stigmator **STEPSIZE** to 3 or 4 on the CRT display. To make finer adjustments, go less under focus (larger diffuse ring) and reduce stigmator **STEP SIZE** to 2 or 3.

- Repeat the process at progressively higher magnifications and smaller focus step sizes.
- You may find it helpful to insert and centre an objective aperture to increase image contrast (see “INSERTING AND CENTRING OBJECTIVE APERTURE” p. 25).
- Once objective astigmastism is corrected, press **STIG** button in **MULTIFUNCTION** panel to deselect STIGMATOR control.

CORRECTING OBJECTIVE ASTIGMATISM
METHOD C: Focused beam, off eucentric

This method is possibly the least sensitive/accurate method for correcting objective astigmatism. However, it can be very useful for crystalline specimens that are lacking in amorphous regions or small round features (e.g. metal alloys).

- Ensure that the region of interest is at eucentric height (see p. 15).
- **FOCUS** the specimen to minimum contrast at **MAGNIFICATION** of e.g. 40000x.

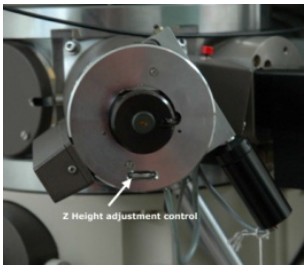
- Using **INTENSITY**, focus the beam to a spot with the smallest possible diameter. **FINE** control may be needed.

- Press **STIG** in the **MULTIFUNCTION** panel to select the **STIGMATOR CONTROL** page on the CRT. Check that **OBJ** is selected on the CRT display. **MULTIFUNCTION X and Y** now control the objective stigmators.

- Making sure that the goniometer tilt is locked (black lever down), rotate the height adjustment control a small amount.

  Rotating it away from you, you should see a diffraction pattern appear and grow in the image of the sample.

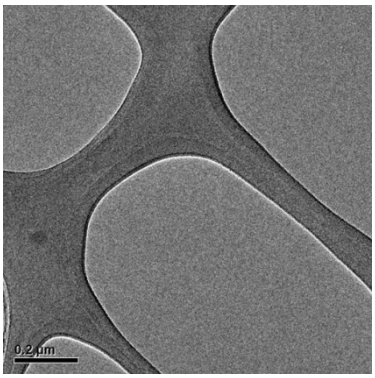
  Rotating it towards you, you should see a disk of uniform intensity appear.



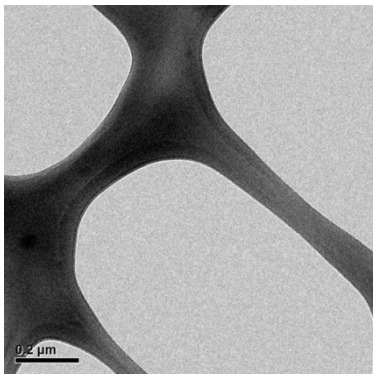
- The circularity of this disc is controlled by objective astigmatism. Adjust the **MULTIFUNCTION X and Y** dials (one at a time) to make the disc as circular as possible. Change stigmator **STEPSIZE** to increase/decrease stigmator sensitivity as required.
- Once objective astigmastism corrected, **return specimen to eucentric height** and press **STIG** button in **MULTIFUNCTION** panel to deselect STIGMATOR control.

INSERTING AND CENTRING OBJECTIVE APERTURE

Use of the **OBJECTIVE** aperture is very important to taking good BF images of your sample. It improves contrast, and further eliminates shadow images from strongly diffracting specimens. For example:



No objective aperture

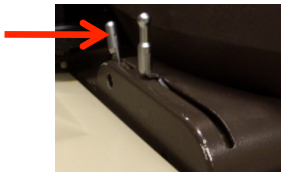


With objective aperture

- Focus the beam to its smallest diameter using the **INTENSITY**, and centre using beam **SHIFT**. Spread the beam across the screen.

- Insert a **Selected Area** aperture. Select an appropriate size, and centre using aperture controls (see “HOW TO USE APERTURES” p. 28)
- Turn **INTENSITY** a long way clockwise (overfocus) until the image is fairly dark.
- Press the **D** button next to **MAGNIFICATION** to go to DIFFRACTION mode.

- Use **MAGNIFICATION** to choose a suitable camera length (i.e. magnification) of the diffraction pattern, e.g. 230 mm or 330 mm.
- Bring in the small screen using the back lever to the left of the column, and look at the pattern through the binoculars.



- Use **FOCUS** to focus the central, brightest diffraction spot to the smallest diameter. Change **FOCUS STEP SIZE** as required. Centre the bright spot using the **MULTIFUNCTION X and Y** dials to move the pattern. If the pattern is too dark, turn **INTENSITY** anticlockwise.

- Insert the **Objective** aperture and select the desired aperture size. Centre the shadow of the aperture around the bright spot in the diffraction pattern. Good centring is important to obtaining a good image.
- Press the **D** button again to return to imaging mode.
- Remove the **Selected Area** aperture.

OBTAINING A SELECTED AREA DIFFRACTION PATTERN

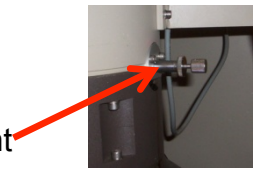
- While in **SA** magnification range, move sample to centre a region of interest.
- Center the beam with **SHIFT X and Y**, and spread across the screen by turning **INTENSITY** clockwise (overfocus).
- Insert a **Selected Area** aperture. Select an appropriate size, and centre aperture with aperture controls (see “HOW TO USE APERTURES” p. 28).
- Turn **INTENSITY** a long way clockwise (overfocus) until the image is fairly dark.
- Press the **D** button next to **MAGNIFICATION** to go to DIFFRACTION mode.

- Select desired **CAMERA LENGTH** using **MAGNIFICATION** dial.
- If it is inserted, remove the **Objective** aperture.
- Adjust the intensity of the **diffraction pattern** to a suitable level by turning the **INTENSITY** dial clockwise/anticlockwise to converge or spread the beam on the sample, and so make the pattern lighter/darker.
- Centre the **diffraction pattern** using the **MULTIFUNCTION X and Y** dials to move the pattern.

- Turn **FOCUS** clockwise/anticlockwise to change focus of diffraction spots. Are the spots astigmatic (i.e. oval) when out of focus? If so, press **STIG** in **MULTIFUNCTION** controls, and correct **DIFF** astigmatism with **MULTIFUNCTION X and Y** dials. Once corrected, press **STIG** again to deselect stigmator control.

- Focus the **diffraction pattern** spots to fine points using **FOCUS** dial.

- If you want to record the diffraction pattern on the **CCD camera**, the diffraction spots are very bright so **be very careful to avoid saturating the camera**. Reduce both intensity of pattern and image acquisition time appropriately. Insert the **mechanical beam stopper** and adjust to cover the central bright spot (stopper control to the right of viewing screen.)



Next, on CRT, select **MODES** then **DET CONF** and select **BF/DF** (or **TV-SYST**). Press **READY** (button at bottom right of CRT) and select **HR-TEM** to return to main CRT page. Then follow CCD instructions to take image of the diffraction pattern (p. 30).

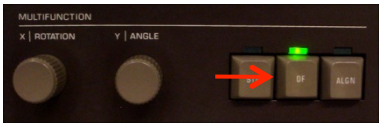
- When finished, press **D** button again to return to **SA** imaging mode.

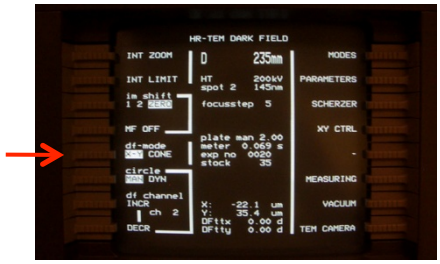
DARK FIELD IMAGING

A quick guide to the using the DARK FIELD controls
(but not dark field theory!)

- Tilt your specimen using the α and β tilts until you obtain the desired tilt condition in the **selected area diffraction pattern** (p. 26) – for example, a two-beam condition.
- In diffraction mode, while using the binoculars, centre the undiffracted (0 0 0) beam in the middle of the small fluorescent screen using **MULTIFUNCTION X and Y**.

- In the **MULTIFUNCTION** panel, press **DF** to select the **HR-TEM DARK FIELD** on the CRT. Check that **df-mode** is set to **X-Y** on the CRT:





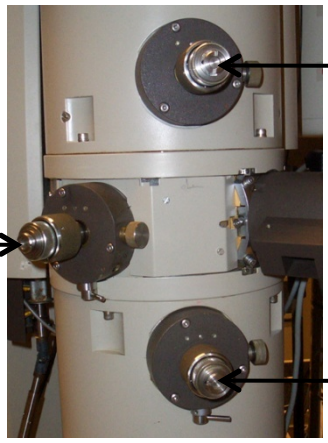
- With **DF** mode selected **MULTIFUNCTION X and Y** dials now control beam tilt. Turn the dials until your chosen reflection is centred in the same place that the undiffracted beam had been previously.

- Turn **DF** mode off to check the coincidence of the undiffracted beam in bright field with the diffracted beam in dark field. Refine their coincidence by repeating the above steps.
- Once their coincidence is good, while in bright field mode, insert an objective aperture and centre on the undiffracted beam. Go to **DF** and check that diffracted beam is well-centred in the aperture.

- Press the **D** button to return to imaging mode. Remove any selected area aperture that is inserted.
- Now, when **DF** is selected in the **MULTIFUNCTION** panel, a dark field image will be obtained. When it is not selected, a bright field image is obtained.

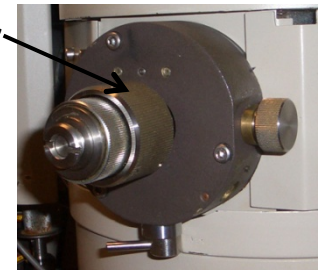
HOW TO USE APERTURES

- The CM12 has three sets of movable apertures on the column:

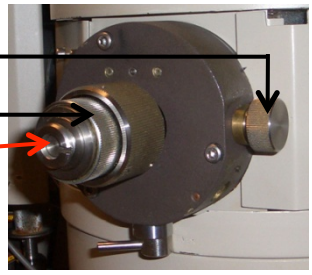


- The apertures can be adjusted in size and centred using their controls

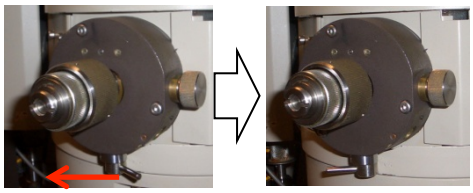
- Aperture size is changed by turning the **large metal cylinder** on the front until another aperture clicks into place:
  - turning **clockwise** brings in a **smaller** aperture
  - turning **anticlockwise** brings in **a larger** aperture
  Each aperture has a choice of 4 sizes.



- Once aperture size is selected, the aperture is centred by turning the two **smaller metal dials**: one on the front, the other on the right.
- Do not touch the outer part with flats for a screwdriver



- Insert the objective or selected area apertures when you need them, by pushing the lever at the bottom of the aperture control to the left.



- The condenser aperture is permanently in. Normally the condenser aperture is left in the second largest size.

- *ALWAYS REMOVE THE **OBJECTIVE** AND **SELECTED AREA** APERTURES AT THE END OF YOUR SESSION BY PUSHING THEIR LEVERS TO THE RIGHT*

FINDING THE BEAM

- Remove the screen cover from the glass window
- Can you see the beam? – i.e. is there green intensity on the fluorescent screen?

- **If you cannot see the beam, try:**

LEFT HAND CONTROL PANEL

- Expanding/contracting beam by turning **INTENSITY** clockwise or anticlockwise. (If you hear a beep, you have reached the limit so turn the other way.)

RIGHT HAND CONTROL PANEL

- Reducing magnification by turning **MAGNIFICATION** (the outer knob) anticlockwise, e.g. in to **LM** mode (magnification mode indicated on the CRT screen).

STAGE CONTROLS

- In case you are on a thick part of the specimen, or on a grid bar, try moving the **X, Y** sample stage controls.

APERTURES

- Check **objective** and **selected area** apertures are out (levers pushed to the right).

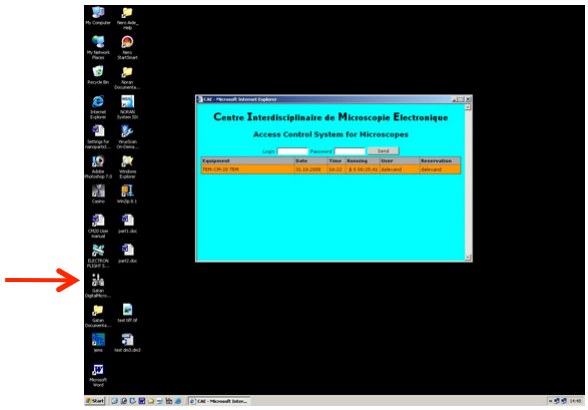
- Once you have found the beam, increase **MAGNIFICATION** to **M** or **SA** mode. Turn **INTENSITY** to focus the beam to a small, bright spot. Centre this spot on the fluorescent screen using the beam **SHIFT X and Y** on the right hand control panel.

USING THE CCD CAMERA

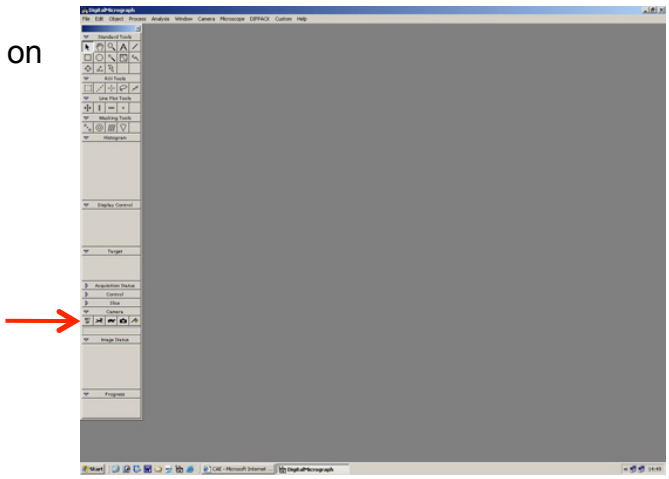
The majority of users use the Gatan 1024x1024 pi CCD camera to take their images. For basic camera use, follow these instructions.

- On top of the left hand control panel, find the camera control box. Turn on **COMPUTER** control of **CAMERA** (switch up). (PELTIER COOLER and AUTO SHUTTER should already be on.)

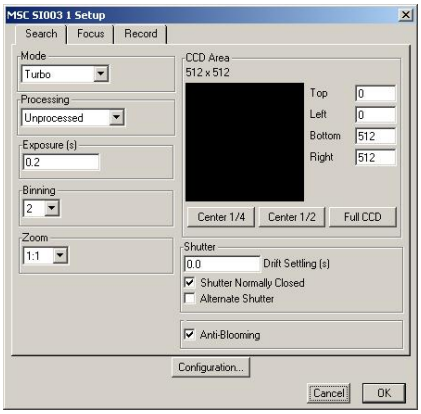
- On the PC computer, open up **Gatan DigitalMicrograph** by double-clicking the desktop icon.

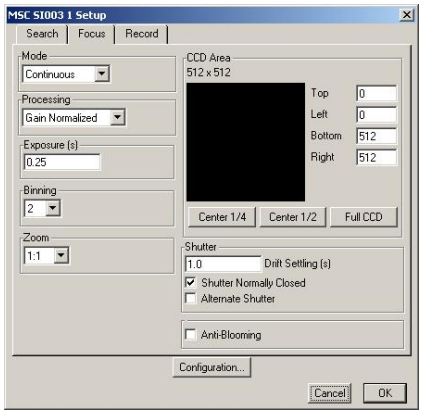


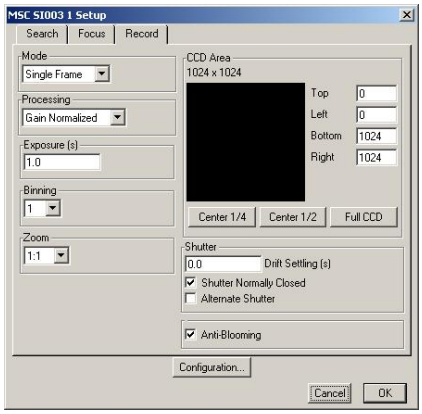
- Once **DigitalMicrograph** has opened, click on **SET UP** to check the camera settings.



- In the **SET UP** window, look at the settings in the **Search**, **Focus** and **Record** tabs. Typical settings are shown below, with Exposure time appropriate to when the beam intensity is spread across the fluorescent screen. **Be careful Exposure time is not too high!** **Binning** (Search, Focus) allows shorter acquisition times for the same beam intensity at the expense of reduced camera resolution. For instance, 2x binning produces a 512 x 512 pi image. Such a 2x binning image will only need a quarter of the acquisition time of a 1024 x 1024 pi 1x binning image for an equivalent counts per pixel in the acquired image.



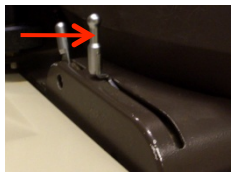




- On the microscope, move the specimen until the region of interest is in the middle of the fluorescent screen. Adjust **MAGNIFICATION** so that this region is slightly smaller than the outer of the circles printed on the centre of the screen, to match the field of view of the CCD camera.

- Turn **INTENSITY** clockwise (i.e. overfocus) so that the beam is spread to about the same diameter as the fluorescent screen.

- Pull forwards the front of the two levers to the left of the glass viewing window to lift the fluorescent screen up and expose the CCD camera.



- In **DigitalMicrograph**, select **Search** mode (hare/rabbit icon) or **Focus** mode (tortoise icon) to start continuous imaging.
- Window opens asking **"Insert 'MSC S1003 1' camera?"**. Select **"Yes".**
- Window opens asking for magnification. Enter the magnification given on the CRT screen. Make sure the fluorescent screen is up, since this changes the effective magnification.
- Adjust image to your satisfaction (e.g. focus, sample position). Click on **Record** (camera icon) to take a single frame at higher image resolution. Save image with **File**, **Save/Save as**.

  Note: if you save image as a **.tif** file, the calibration information is lost and the scale bar is burned in to the image. Therefore we recommend *first* saving as **.dm3** and *then* as **.tif** since the image calibration is preserved in the **.dm3** file.

DigitalMicrograph/CCD Camera tips and trouble-shooting

- Higher beam intensity on the camera/longer acquisition times/greater binning produce images with higher signal-to-noise.
- To check the signal counts recorded on your image, first make sure that the image is the front-most window open in DigitalMicrograph. Hover the mouse cursor (arrow shape cursor style selected) over the image and look in the "**Image Status**" box on the left of the screen. In this "**Image Status**" box there will be a number entitled "**Value**" that indicates the number of counts for that particular pixel. (The brighter the image region, the greater the number of counts) For a good image, the brightest parts of the image should typically have a "**Value**" in the region of 2000 to 8000.
- **Be careful not to oversaturate** the camera with use of e.g. too high a beam intensity. Over-saturation is avoided by keeping the counts per pixel to less than ~10000. Oversaturation will be obvious because the over-saturated image shows the honeycombe structure of the CCD camera, rather than the contrast from your imaging region.
- **Oversaturation is a particular concern when recording diffraction patterns.** If you wish to record diffraction patterns, carefully follow the instructions on p. 30. Alternatively, use photographic imaging plates.
- When imaging on continuous acquisition (Search/Focus), pressing the up arrow on the computer keyboard doubles the acquistion time, and pressing the down arrow halves it.

- **Trouble-shooting:** If the images from the CCD camera appear to contain artefacts not present in your sample, click the "**Camera**" drop-down menu in the task bar at the top of the DigitalMicrograph window, and select "**Remove Dark References**". On the camera control unit to the left of the microscope column, check **SHUTTER** is on **BF/DF**.

  If you suspect the images still contain artefacts, move your sample so that the CCD camera images only vacuum (i.e. a hole). The image of the vacuum should be flat, grey noise with no defined features or contrast variations. If this is not the case, please contact one of the responsibles or CIME personnel.

  We want you to be taking the best quality images possible, and therefore do not want you to be taking images with obvious (and solvable) CCD camera artefacts! Therefore do not be afraid to contact us!

END OF SESSION: SHUTTING DOWN

It is important when you end your session to turn off the gun and bring the microscope back to its standard conditions.

- Turn the **MAGNIFICATION** to **40000x** in the TEM (not TEM low-dose) mode.
- Remove **Objective and Selected Area apertures** if they are inserted.
- On the **PC computer**, close **DigitalMicrograph** program. On CCD camera control, set **CAMERA** to **OUT**.

- Go to **CONFIGURATION** page on CRT. Slowly reduce **FILAMENT** to zero, one click every 5 seconds.

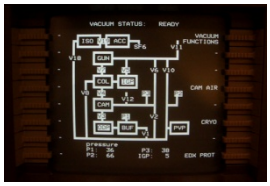
- Go to **PARAMETERS** page on CRT. Select **120 kV**. Return to main CRT page.

- Turn off any buttons on the lower part of both panels (LHS & RHS) that have been left on (i.e. green lights off).

- Follow **specimen exchange procedure (p. 5-7)** to remove your sample. Before removing sample holder, ensure stage positions **X: ~0.0 um, Y: ~ 0.0 um** and **manual (α) tilt is locked at 0°** and, for **double tilt holder, β tilt is at intermediate position**.

  Remove your sample and load empty single tilt holder in to microscope.

- Check **VACUUM STATUS: READY** on **VACUUM** page of CRT.



- If last user in the day, remove liq. N2 dewar and start Cryo cycle on vacuum page
- Pull out the **PANEL DIM** button to turn off the panel lights & turn the **DATA DIM** anti-clockwise to dim the CRT screen.

- Put window covers back on the glass viewing windows.
- **Log out** of your session on the PC computer.